Mycobacterial arthritis and synovitis in Painted reed frogs (*Hyperolius marmoratus*)

Michelle Barrows[1], Katja Koeppel[2], Anita Michel[2], Emily Mitchell[3]

[1]Bristol Zoological Society, Clifton, Bristol BS8 3HA.

[2]Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.

[3] National Zoological Gardens of South Africa, Pretoria, South Africa

Correspondence: Michelle Barrows, Bristol Zoo Gardens, Clifton, Bristol BS8 3HA.

+44 (0)117 9747339 mbarrows@bristolzoo.org.uk

+

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Keywords: Painted reed frog; *Hyperolius marmoratus; Mycobacterium chelonae*; *Mycobacterium abscessus;* arthritis; synovitis

Summary:

Several species of atypical mycobacteria have been isolated from wild and captive amphibians. In captive anurans cutaneous and visceral mycobacteriosis are common and can result in significant mortality, particularly when animals are immunocompromised. Mycobacterial arthritis and synovitis have rarely been reported in amphibians. We describe 20 cases in painted reed frogs, *Hyperolius marmoratus* which presented with cachexia, limb paresis or paralysis or 'spindly leg syndrome'. Histopathology revealed multifocal histiocytic to granulomatous synovitis affecting appendicular, rib or spinal intervertebral joints. Periarticular granulomata, granulomatous cellulitis and skeletal muscle atrophy, necrosis and degeneration were also present. In one case granulomatous spinal osteomyelitis was recorded. Ziehl-Neelson stains showed large numbers of acid-fast bacteria in macrophages and histiocytes. The mycobacterial isolates obtained from culture were identified as members of the *Mycobacterium chelonae* complex (either *M. chelonae* or *M. abscessus*). This was confirmed by 5'16sRNA sequencing. In 17 cases mycobacterial lesions were present only in the joints and skeleton highlighting the importance of not ruling out mycobacterial infection on the basis of lack of cutaneous or visceral lesions.

Mycobacteriosis is an important infectious disease in amphibians. Species of atypical mycobacteria identified from anurans, caecilians and caudates include *M. abscessus (*Mok and Carvalho, 1984), *M. avium* (Chai *et al*., 2006), *M. chelonae (*Green *et al.*, 2000), *M. fortuitum* (Darzins, 1952*), M.gordonae* (Kirsch *et al*., 2008; Sánchez-Morgado *et al*., 2009), *M.liflandii* (Rowlatt and Roe, 1966; Godfrey *et al*., 2007), *M. marinum* (Clark and Shepard, 1963; Moraes, 1999; Maslow *et al*., 2002; Pizzi and Miller, 2005; Cannon *et al*., 2006), *M. szulgai* (Chai *et al*., 2006), *M. ulcerans* (Portaels *et al*., 2001, Mve-Obiang *et al*., 2005) and *M. xenopi* (Taylor *et al*., 2001). However Martinho and Heatley (2012) in a review of amphibian mycobacteriosis caution that with improvements in molecular evaluation techniques, previous diagnoses may not reflect current classification of mycobacterial species.

Although mortality rates associated with mycobacteriosis are generally low in captive anurans, it has been reported as the leading cause of death in some research frog colonies (Fremont-Rahl *et al*., 2011; Chai, 2012). Mature amphibians are affected more often than tadpoles and mycobacterial infection is often subclinical, with disease developing mainly in immunocompromised animals (Clark and Shepard, 1963; Ramakrishnan *et al*., 1997; Ferreira *et al*., 2006; Chai, 2012; Martinho and Heatley, 2012). Anuran families known to be susceptible to mycobacteriosis include Bufonidae, Hylidae, Leptodactylidae, Pipidae, Pseudidae, Dendrobatidae and Ranidae with most infections reported in African clawed frogs (*Xenopus* species) (Green *et al*., 2000; Trott *et al*., 2004; Godfrey *et al*., 2007; Suykerbuyk *et al*., 2007; Reavill and Schmidt, 2012).

Mycobacteria are ubiquitous in soil and aquatic environments (Chai, 2012, Martinho and Heatley, 2012) and have been isolated from both wild anurans and their environments (Mok and Carvahlo, 1984; Willson *et al*., 2013; Garchitorena *et al*., 2014). The mycobacterial

species affecting amphibians are water-borne and can infect frogs through skin lesions or wounds. Ingestion is also a potential route of infection in both tadpoles and adults (Nonidez and Kahn, 1937). Vectors such as protozoa may transmit mycobacteria to amphibians (Gauthier and Rhodes, 2009). As in other species, infection results in chronic granulomatous inflammation. Granulomata may be irregular and poorly demarcated, or large and encapsulated. Early granulomata are composed of mostly epithelioid macrophages and progress to form encapsulated foci consisting of variable numbers of macrophages, lymphocytes, epithelioid cells, fibroblasts and melanocytes surrounding a necrotic centre (Asfari 1988; Bouley *et al*., 2001; Trott *et al*., 2004). Early granulomata have been confused for neoplastic lesions such as lymphosarcoma (Asfari 1988; Green, 2001), however confirmation of the diagnosis is simple as they usually contain large numbers of acid-fast bacilli. Caseation is a variable and unreliable feature and mineralisation and cavitation are not reported (Green, 2001). Cutaneous and visceral mycobacteriosis are the most common forms reported in anurans. In the visceral form granulomata develop in the liver, spleen, kidneys and gastrointestinal tract. In the cutaneous form skin lesions include hyperaemia, petechiae and ulcers, as well as miliary grey or whitish nodules (Shiveley *et al*., 1981; Taylor *et al*., 2001; Trott *et al*., 2004; Ferreira *et al*., 2006; Hill *et al*., 2010). Clinical signs vary, with some individuals asymptomatic and others presenting with sudden death. Weight loss, anorexia and emaciation may occur but are not consistent clinical signs. Other reported clinical signs include coelomic swelling, bloating and abnormal buoyancy, subcutaneous oedema, ulcerative or nodular dermatitis, ataxia and mucopurulent nasal and oral secretions. A pathological tibial fracture attributed to mycobacterial infection has been described in a marine toad (*Bufo marinus*) (Fitzgerald *et al*., 2004). Mycobacterial arthritis however has rarely been reported in amphibians. There is an anecdotal report of mycobacteriosis causing spinal lesions in Wyoming toads (*Bufo baxteri*) and mountain yellow-legged frogs (*Rana*

muscosa) (Pessier, 2014) and a description of mycobacterial infection affecting the cervical vertebrae and a tibiotarsal joint in an American toad (*Bufo americanus*) (Done *et al*., 1993). In this paper we report mycobacterial arthritis and synovitis affecting both the appendicular and axial skeleton of painted reed frogs, *Hyperolius marmoratus*, a small insectivorous anuran species from sub-Saharan Africa.

Thirty six adult painted reed frogs were collected from the wild in Mpumalanga province, South Africa in order to initiate a captive breeding programme. The purpose of this project was to develop husbandry protocols for the painted reed frog as an analogue species for future maintenance of a population of the critically endangered Pickersgill's reed frog (*Hyperolius pickersgilli*). Adults were housed in 5 litre transparent plastic jars with a paper towel substrate and 8ml of reverse osmosis (RO) water or in plastic tanks (320 x 220 x 250mm) containing smooth aquarium gravel and moss. The tanks were housed on racks with a recirculating RO water system connected to a shared biological filtration system as described by Van der Spuy and Krebs (2008). Diet consisted of appropriately sized crickets, fruit flies, silkworms and termites all dusted with calcium powder. Tadpoles were housed in glass tanks (912 x 325 x 325mm) with an air pump and canister filter with the inlet spray bar placed on the wall so that water flowed down the glass into the tank. Tadpoles were fed on spirulina tablets and cos lettuce. Painted reed frogs were successfully bred to the F3 generation in these systems.

The study animals consisted of 20 frogs which were found dead or humanely euthanased due to cachexia, limb paresis, paralysis, deformity or 'spindly leg syndrome', or because they were in contact with other affected frogs. They included some wild caught adults and some captive bred frogs from the F1 and F3 generations. The first cases occurred in wild caught frogs 14 months after capture. Frogs were preserved in 10% buffered formalin and multiple

serial sections made through the entire carcass for histopathology. In addition joint samples from nine of the frogs were submitted as fresh unfixed tissue for mycobacterial culture. Tissues from each of these nine were homogenised and decontaminated in equal volumes of 1% hydrochloric acid and 2% sodium hydrochloride followed by neturalisation in double distilled sterile water, centrifugation and inoculation on Loewenstein-Jensen medium containing glycerol. Medium slopes were incubated at 25◦C, 37◦C and 42◦C and monitored for colony growth twice per week. Mycobacterium colonies were subjected to Ziehl-Neelsen staining and acid-fast isolates were subcultured and subjected to biochemical characterisation including 3-day arylsulfphatase acitivty test and nitratase reductase activity. 5'-16S ribosomal ribonucleic acid (rRNA) PCR-sequencing was carried out on two isolates (Rogall *et al*., 1990).

The most significant findings on histopathology were in the appendicular and spinal intervertebral joints. All the frogs had multifocal histiocytic to granulomatous synovitis affecting at least one and often several distal appendicular joints, rib joints or spinal intervertebral joints (Figures 1-3 with a normal joint for comparison). The synovial membranes were greatly expanded by aggregates of large foamy macrophages mixed with a few heterophils. In one case there was osteolysis of distal bone epiphyses. In addition, periarticular granulomata consisting of loose aggregates of large, reactive macrophages mixed with fewer lymphocytes and occasional necrotic heterophils were present surrounding the joint cartilages in affected joints, sometimes obliterating the joint space and extending into the subcutis and skeletal muscle. Small granulomata in the spinal canal at intervertebral joints, sometimes compressed the spinal cord. In one case granulomatous spinal osteomyelitis was present. Rostral spinal column bone and cartilage was multifocally necrotic and replaced by loose aggregates of macrophages (Figure 4). Ziehl-Neelson (ZN) stains showed small to large numbers of acid fast bacteria in macrophages in synovial

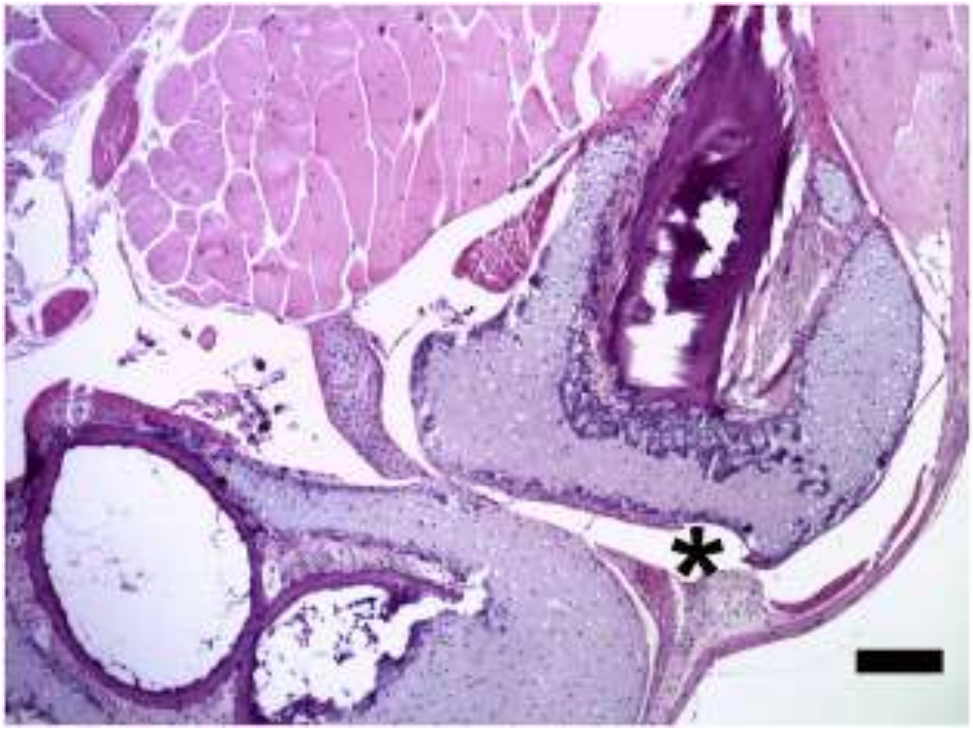

Figure 1: Normal appendicular limb joint in a painted reed frog. Note clear articular space between cartilage caps (*). Haematoxylin and eosin, Bar = 0.3mm

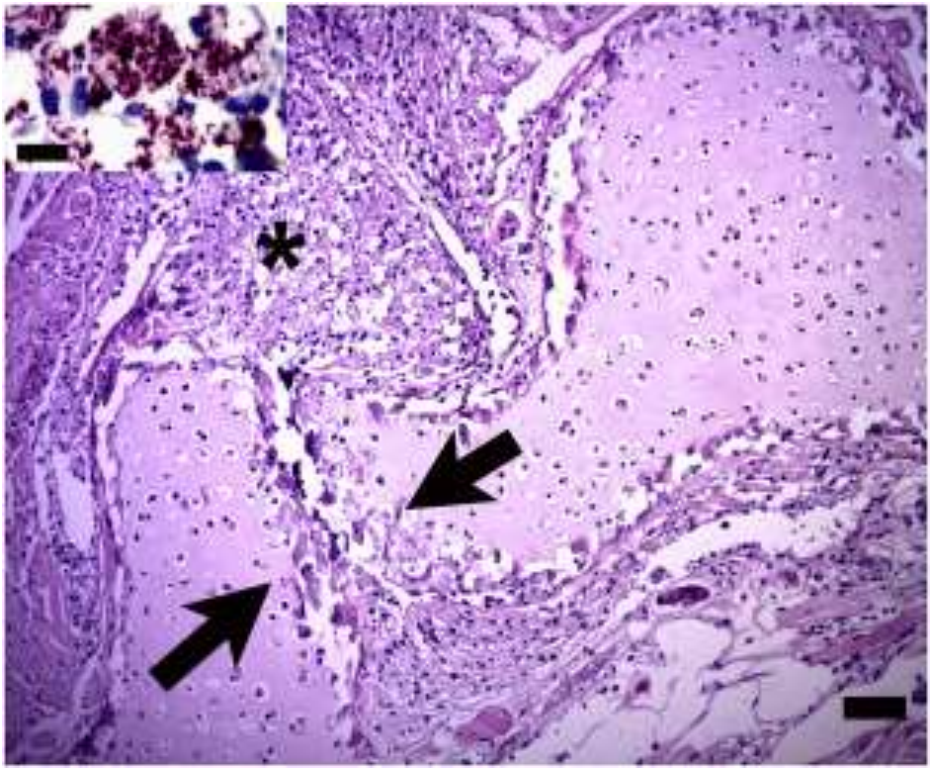

Figure 2: Histiocytic arthritis in stifle joint of a painted reed frog. Loose aggregates of large foamy macrophages (*) containing myriad acid fast organisms (Ziehl Neelsen, insert) fill the joint space between cartilage caps which show degeneration and necrosis (arrows). Haematoxylin and eosin, Bar = 0.3mm

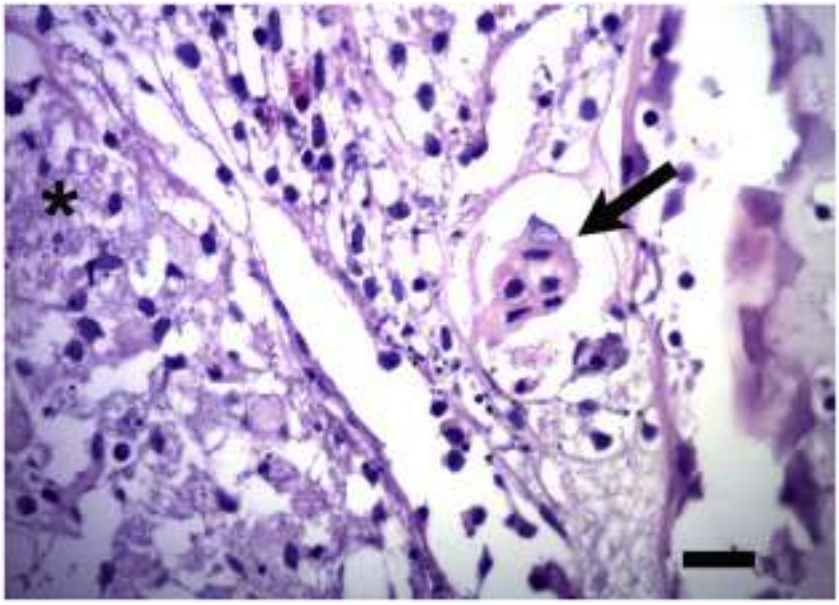

Figure 3: Histiocytic arthritis in stifle joint of a painted reed frog. Loose aggregates of large foamy macrophages (*) and one multinucleate giant cell (arrow) fill the joint space. Haematoxylin and eosin, Bar = 10 µm

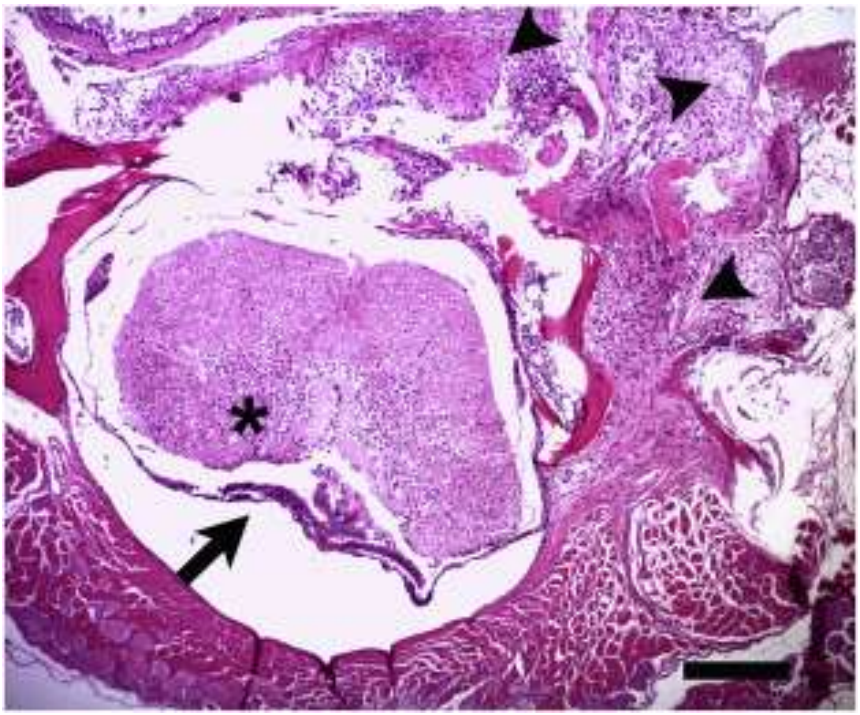

Figure 4: Histiocytic osteomyelitis, cellulitis and myositis (arrows) of the spinal column of a painted reed frog. Large numbers of macrophages, mixed with fewer lymphocytes and plasma cells infiltrate and expand the spinal cord (*), dura mater (arrow) and surrounding bone and skeletal muscle (arrowheads). Haematoxylin and eosin, Bar = 0.8mm

membranes, cellulitis, skeletal muscle and spinal canal (Fig 2, insert). One frog had moderate diffuse heterophilic, histiocytic and lymphocytic interstitial nephritis with acid-fast organisms in renal histiocytes, two had multifocal hepatic granulomata and in another case, small to medium numbers of acid-fast bacterial rods consistent with mycobacteria occurred in macrophages in the meninges. Associated histopathology showed hepatic atrophy and lipidosis, skeletal muscle atrophy and pancreatic zymogen granule depletion. In addition, all frogs displayed prominent hepatic melanomacrophage hyperplasia. Other additional findings, each seen in only one or two frogs included acute renal tubular necrosis, renal haemosiderosis, diffuse membranous glomerulonephritis, microfilarial hepatitis, hepatic karyomegaly, meningeal melanosis, cerebral protozoa, mild bone marrow myelopoiesis, skeletal muscle nematodes and intestinal mucosal associated lymphoid hyperplasia. Apart from congestion all other tissue samples were unremarkable histologically. Mycobacterium isolates were identified as *Mycobacterium chelonae-abscessus* complex organisms based on their non-pigmented colony appearance, rapid growth rate (<7 days), ability to grow at 25◦C, arylsulfatase activity and negative nitrate reduction. 5'-16S rRNA PCR-sequencing was successful on one of two isolates submitted and confirmed it to be a member of the *Mycobacterium chelonae* complex (either *M. chelonae* or *M. abscessus*).

Mycobacterial arthritis and synovitis have rarely been reported in amphibians. Cutaneous and visceral lesions were conspicuous by their absence in this case series. Death in those frogs that died spontaneously was likely caused by the metabolic effects of inadequate food intake, due to locomotor dysfunction as a result of involvement of the axial and/or appendicular skeleton. Hepatic lipidosis and pancreatic zymogen granule depletion were likely attributable to anorexia. Some affected juvenile frogs presented as cases of ‘spindly leg syndrome’, a relatively common presentation in anurans characterized by limb

deformities, muscle atrophy and sometimes absence of limb bones (Hakvoort *et al*., 1995; Claunch and Augustine, 2015). The aetiology is unknown but nutrition, genetics, environment and trauma are potential contributing factors (Marlett *at al*., 1988; Wright, 2001). This case series highlights the need to consider mycobacterial infection as a differential diagnosis for muscle atrophy of the limbs in anurans.

Mycobacteriosis has been reported in several wild anuran species with prevalences varying from 2% in common lesser toads (*Bufo granulosus*) and 19.6% in four-eyed frogs (*Pleurodema cinerea* and *P.marmoratus*) to 100% in South American bullfrogs (*Leptodactylus pentadactylus*) from an urban area of Brazil (Darzins, 1952; Machicao and LaPlaca, 1954; Mok and Carvahlo, 1984). It is therefore possible that the painted reed frogs were infected with mycobacteria when they were caught, however given that the first mortalities in wild caught adults occurred 14 months after capture and that cases also occurred in F1 and F3 generations, it is more likely that the infection was contracted in captivity. The most likely source was the water; *M. liflandii* has been isolated from water in an epizootic of mycobacteriosis in *Xenopus tropicalis* and an atypical mycobacterium was found in tap water used to house *Xenopus laevis* (Kirsch *et al*., 2008; Chai, 2012). *M. chelonae* and *M. fortuitum* have also been isolated from water samples taken from amphibian breeding grounds in Brazil (Mok *et al*., 1987). Ingestion is one potential route of entry. Entry through skin lesions is another and although no skin lesions were present in any of the frogs at post mortem examination, one individual had nematode larvae in the muscle indicating that larval penetration of the skin might have occurred suggesting a potential route of invasion for mycobacteria. Once mycobacteria had entered the body, haematogenous spread to the joints may have occurred, as suspected in a sea turtle with mycobacterial arthritis (Greer *et al*.,2003). Lymphatic spread is also possible. In one study, after

experimental infection of tadpoles, mycobacteria were assumed to have spread within macrophages migrating via the lymphatics, since none were detected in serial sections of the heart and major blood vessels (Nonidez and Kahn, 1937).

Mycobacterial arthritis and/or osteomyelitis has been reported in humans, horses and reptiles (Kelly *et al*., 1972; Shu *et al*., 2009; Gardam and Lim, 2005; Greer et al., 2003; Hewes *et al*., 2005; Kramer, 2006; Winthrop and Iseman, 2013). In all species, immunosuppression is a significant predisposing factor. Immunosuppressive drugs used to treat rheumatoid arthritis and infection with human immunodeficiency virus have been implicated in human cases and a horse with septic arthritis caused by *M.avium* complex had previously received multiple cortisol injections into the affected joint (Shu *et al*., 2009; Gardam and Lim, 2005; Hewes *et al*., 2005; Winthrop and Iseman, 2013). A bearded dragon (*Pogona vitticeps*) with osteomyelitis due to atypical mycobacteriosis was assumed to be immunocompromised due to inadequate husbandry (Kramer, 2006). Immunosuppression is well recognized as a predisposing factor for mycobacteriosis in amphibians and it is likely to have played a role in this case series (Clark and Shepard, 1963; Ramakrishnan *et al*., 1997; Ferreira *et al*., 2006; Chai, 2012; Martinho and Heatley, 2012). Immunosuppression was also thought to be a factor in 15 cases of spinal arthropathy due to the bacterium *Ochrobactrum anthropi* in cane toads (*B.marinus*) (Shilton *et al*., 2008).

The reason for the unique site predilection of the mycobacterial infection identified in these painted reed frogs is unclear. There may be a genetic predisposition or the particular mycobacterial species involved may have a predisposition for joint tissues. It is important that mycobacterial infection is not ruled out on the basis of lack of cutaneous or visceral

lesions in anurans and that husbandry protocols for all captive amphibians are designed to minimise stress and consequent immunosuppression.

Acknowledgements:

We would like to thank Professor NC Gey van Pittius (University of Stellenbosch) for assistance with mycobacterial 5'16s rRNA sequencing and speciation and the National Research Foundation and National Zoological Gardens of South Africa for logistical and administrative support. We also appreciate the technical expertise provided by the pathology laboratory staff in the Department of Paraclinical Studies, Faculty of Veterinary Science. We would also like to acknowledge the animal collections staff at Johannesburg Zoo who cared for the frogs.

References

Asfari M (1988) Mycobacterium-induced infectious granuloma in *Xenopus*: histopathology and transmissibility. *Cancer Research*, **48**, 958–963.

Bouley D, Ghori N, Mercer K, Falkow S, Ramakrishnan L (2001). Dynamic nature of host-pathogen interactions in *Mycobacterium marinum* granulomas. *Infection and Immunity*, **69**, 7820–7831.

Cannon C, Linder K, Brizuela B, Harvey S (2006) Marked swelling with coalescing ecchymoses of the lower mandible in a *Xenopus laevis* frog. *Laboratory Animals*, **35**, 19 –22.

Chai N, Deforges L, Sougakoff W, Truffot-pernot C, De Luze A *et al*. (2006) *Mycobacterium szulgai* infection in a captive population of African clawed frogs (*Xenopus tropicalis*). *Journal of Zoo and Wildlife Medicine*, **37**, 55–58.

Chai N (2012) Mycobacteriosis in Amphibians. In Fowler's Zoo and Wild Animal Medicine Current Therapy volume 7. Elsevier Saunders St Louis, Missouri pp224-230.

Clark H, Shepard C (1963) Effect of environmental temperatures on infection with *Mycobacterium marinum* (Balnei) on mice and a number of poikilothermic species. *Journal of Bacteriology*, **86**, 1057–1069.

Claunch N and Augustine L (2015) Morphological description of spindly leg syndrome in golden mantella (*Mantella aurantiaca*) at the Smithsonian National Zoological Park. *Journal of Herpetological Medicine and Surgery*, **25**, 72-77.

Darzins E (1952) The epizootic of tuberculosis among the gias in Bahia. *Acta Tuberculosea Scandinavica*, **26**, 170–174.

Done L, Willard-Mack C, Ruble G, Cranfield M (1993) Diagnostic exercise: ulcerative dermatitis and cellulites in American toads. *Laboratory Animal Science*, **43**, 619-621.

Ferreira R, Fonseca L, Afonso A, da Silva M, Saad M *et al*. (2006) A report of mycobacteriosis caused by *Mycobacterium marinum* in bullfrogs (*Rana catesbeiana*). *Veterinary Journal*, **171**,177–180.

Fitzgerald S, deMaar T, Thomas J, Berry D (2004) Pathologic limb fracture attributed to mycobacterial infection in a marine toad, *Bufo marinus*, with systemic mycobacteriosis and chromomycosis. *Journal of Herpetological Medicine and Surgery*, **14**, 19-23.

Fremont-Rahl J, Ek C, Williamson H, Small P, Fox J *et al*. (2011) *Mycobacterium liflandii* outbreak in a research colony of *Xenopus (Silurana) tropicalis* frogs. *Veterinary Pathology*, **48**, 856–867.

Garchitorena A, Roche B, Kamgang R, Ossomba J, Babonneau J *et al*. (2014) *Mycobacterium ulcerans* Ecological Dynamics and Its Association with Freshwater Ecosystems and Aquatic Communities: Results from a 12-Month Environmental Survey in Cameroon. *PLoS Neglected Tropical Diseases*, doi: 10.1371/journal.pntd.0002879

Gardam M, Lim S. (2005) Mycobacterial osteomyelitis and arthritis. *Infectious Disease Clinics of North America*, **19**, 819-830.

Gauthier D and Rhodes M (2009) Mycobacteriosis in fishes: a review. *Veterinary Journal*, **180**, 33-47.

Godfrey D, Williamson H, Silverman J, Small P (2007) Newly identified *Mycobacterium* species in a *Xenopus laevis* colony. *Comparative Medicine*, **57**, 97–104.

Green S, Lifland B, Bouley D, Brown B, Wallace R *et al*. (2000) Disease attributed to *Mycobacterium chelonae* in South African clawed frogs (*Xenopus laevis*). *Comparative Medicine*, **50**, 675–679.

Green D (2001) Pathology of amphibia. In: *Amphibian medicine and captive husbandry*. K Wright, B Whitaker, Eds., Krieger Publishing, Malabar, Florida, pp. 401-485.

Greer L, Strandberg J, Whitaker B (2003) *Mycobacterium chelonae* osteoarthritis in a Kemp's ridley sea turtle, *Lepidochelys kempii*. *Journal of Wildlife diseases*, **39**, 736-741.

Hakvoort J, Gouda E, Zwart P (1995) Skeletal and muscular underdevelopment (SMUD) in young tree frogs (Dendrobatidae). *Proceedings of the 5th International Symposium of Pathology of Reptiles and Amphibians*, Alphen a Rjin, The Netherlands, pp. 271-275.

Hewes C, Schneider R, Baszler T, Oaks J (2005) Septic arthritis and granulomatous synovitis caused by infection with *Mycobacterium avium* complex in a horse. *Journal of the American Veterinary Medical Association*, **226**, 2035-2038.

Hill W, Newman S, Craig L, Carter C, Czarra J *et al*. (2010) Diagnosis of *Aeromonas hydrophila*, *Mycobacterium* species and *Batrachochytrium dendrobatidis* in an African clawed frog (*Xenopus laevis*). *Journal of the American Association of Laboratory Animal Science*, **49**, 215–220.

Kelly W, Collins J, Farrelly B, Whitty B, Rhodes W (1972) Vertebral Osteomyelitis in a Horse Associated with *Mycobacterium tuberculosis var. Bovis* (*M. Bovis*) Infection. *Veterinary Radiology & Ultrasound*, **13**, 59-69.

Kirsch P, Nusser P, Hotzel H, Moser I (2008). *Mycobacterium gordonae* as potential cause of granulomatous lesions of the toe tips in the South African clawed frog (*Xenopus laevis*). Berliner und Münchner Tierarztliche Wochenschrift **121**, 270-277.

Kramer M (2006). Granulomatous osteomyelitis associated with atypical mycobacteriosis in a Bearded Dragon (*Pogona vitticeps*). *Veterinary Clinics of North America Exotic Animal Practice,* **9**, 563-568.

Machicao N, LaPlaca E (1954) Lepra-like granulomas in frogs. *Laboratory Investigation*, **3**, 219-227.

Marlett J, Eichler V, Fellows-Hagemeister M (1998) Spindly leg syndrome in frogs: present knowledge and future directions to elucidate its causes. *International Herpetological Symposium on Captive Propagation and Husbandry*, **11**, 145-150.

Martinho and Heatley (2012) Amphibian Mycobacteriosis. *Veterinary Clinics of North America Exotic Animal Practice* **15**: 113-119.

Maslow J, Wallace R, Michaels M, Foskett H, Maslow E *et al*. (2002) Outbreak of *Mycobacterium marinum* among captive snakes and bullfrogs. *Zoo Biology*, **21**, 233–241.

Mok W, Carvalho C (1984) Occurrence and experimental infection of toads (*Bufo marinus* and *B. granulosus*) with *Mycobacterium chelonae* subsp. *abscessus*. Journal of Medical Microbiology, **18**, 327–33.

Mok W, Carvalho C, Barreto da Silva M. (1987) Host-parasite relationship between amazonian anurans and atypical mycobacteria (*Mycobacterium chelonei* and *M. fortuitum*). *Biotropica*, **19**, 274 –277.

Moraes J, Martins M, Souza V, Moraes F, Souza J (1999) Anatomopathological diagnosis of mycobacteriosis in bullfrogs (*Rana catesbeiana* Shaw, 1802) from Brazilian commercial frog farms. ARS Veterinaria, **15**, 110–114.

Mve-Obiang A, Lee R, Umstot E, Trott K, Grammer T *et al*. (2005) A newly discovered mycobacterial pathogen isolated from laboratory colonies of *Xenopus* species with lethal infections produces a new form of mycolactone, the *Mycobacterium ulcerans* macrolide toxin. *Infection and Immunity*, **73**, 3307–3312.

Nonidez J, Kahn M (1937) Experimental tuberculosis infection in the tadpole and the mechanism of its spread. *American Review of Tuberculosis*, **36**, 191-211.

Pessier A (2014) Infectious diseases of amphibians: it isn't just red leg anymore. In: *Current therapy in reptile medicine and surgery*. D Mader, S Divers, Eds., Elsevier Saunders St Louis, Missouri, pp. 247-254.

Pizzi R, Miller J. (2005) Amputation of a *Mycobacterium marinum*-infected hindlimb in an African bullfrog (*Pyxicephalus adspersus*). *Veterinary Record*, **156**, 747–748.

Portaels F, Chemlal K, Elsen P, Johnson P, Hayman J *et al*. (2001) *Mycobacterium ulcerans in wild animals. Scientific and Technical Review of the Office International des Epizooties* (Paris), **20**, 252–264.

Ramakrishnan L, Valdivia R, McKerrow J, Falkow S (1997). *Mycobacterium marinum* causes both long-term subclinical infection and acute disease in the leopard frog (*Rana pipiens*). *Infection and Immunity*, **65**, 767–773.

Reavill D and Schmidt R (2012) Mycobacterial lesions in fish, amphibians, reptiles, rodents, lagomorphs and ferrets with reference to animal models. *Veterinary Clinics of North America Exotic Animal Practice*, **15**: 25-40.

Rogall T, Flohr T, Böttger E. (1990) Differentiation of *Mycobacterium* species by direct

154 sequencing of amplified DNA. *Journal of General Microbiology*, **136**, 1915-1920.

Rowlatt U, Roe F (1966) Generalized tuberculosis in a South American frog *Leptodactylus pentadactylus*. *Pathologia veterinaria*., **3**, 451–460.

Sánchez-Morgado J, Gallagher A, Johnson L (2009) *Mycobacterium gordonae* infectionin a colony of African clawed frogs (*Xenopus tropicalis*). *Laboratory Animals*, **43**, 300 –303.

Shilton C, Brown G, Benedict S, Shine R. (2008) Spinal arthropathy associated with *Ochrobactrum anthropi* in free-ranging cane toads (*Chaunus [Bufo] marinus*) in Australia. *Veterinary Pathology*, **45**, 85–94.

Shiveley J, Songer J, Prchal S, Keasey M, Thoen C (1981) *Mycobacterium marinum* infection in bufonidae. *Journal of Wildlife Diseases*, **17**, 3–7.

Shu C, Wang J, Yu C, Lee L (2009) Mycobacterial arthritis of large joints *Annals of the Rheumatic Diseases*, **68**,1504-1505.

Suykerbuyk P, Vleminckx K, Pasmans F, Stragier P, Ablordey A *et al*. (2007) *Mycobacterium liflandi* infection in European colony of *Silurana tropicalis*. *Emerging Infectious Diseases*, **13**, 743–746.

Taylor S, Green D, Wright K, Whitaker B (2001). Bacterial diseases. In: *Amphibian medicine and captive husbandry*. K Wright, B Whitaker, Eds., Krieger Publishing, Malabar, Florida, pp 159–180.

Trott K, Stacy B, Lifland B, Diggs H, Harland R *et al*. (2004). Characterization of a *Mycobacterium ulcerans*-like infection in a colony of African tropical clawed frogs (*Xenopus laevis*). *Comparative Medicine* **54**, 309 –317.

Van der Spuy S and Krebs J (2008) Collaboration for amphibian conservation: the establishment of the Johannesburg Zoo Amphibian Conservation Center in South Africa with assistance from Omaha's Henry Doorly Zoo, USA. *International Zoo Yearbook* **42**:165-171.

Willson S, Kaufman M, Merritt R, Williamson H, Malakauskas D *et al*. (2013) Fish and amphibians as potential reservoirs of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer disease. *Infection Ecology and Epidemiology*, 2013; **3**: 10.3402/iee.v3i0.19946.

Winthrop K, Iseman M (2013) Bedfellows: mycobacteria and rheumatoid arthritis in the era of biologic therapy *Nature Reviews Rheumatology* **9**, 524-531.

Wright K (2001) Idiopathic syndromes. In: *Amphibian medicine and captive husbandry*. K Wright, B Whitaker, Eds., Krieger Publishing, Malabar, Florida, pp 239-244.